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Encased Cantilevers for Ultra-Low-Noise Force Spectroscopy of Proteins and Ligand Receptor Complexes

Dominik Ziegler, Paul D. Ashby.
Lawrence Berkeley National Laboratory, Berkley, CA, USA.

The energy landscape of proteins folding and unfolding or of ligand-receptor binding can be elucidated by force spectroscopy. Laser tweezers has excellent force resolution (~10 fN/sqrt(Hz)) but a low force limit, making it poorly suited for protein pulling. Atomic Force Microscopy has a high force limit but poor force resolution (~200 fN/sqrt(Hz)) because of viscous interaction of the water with the cantilever. To reduce viscous damping we developed encased cantilevers in which the cantilever is kept dry. Immersed in the solution, surface tension prevents the liquid from entering into the encasement, and only few microns of the tip protrude to interact with the sample in solution. The length of the free cantilever within the encasement is easily controlled during fabrication, hence, ultra short cantilevers with resonance frequencies >1MHz can be fabricated (Figure1, a and b). Thanks to the low damping we achieve quality factors of >100 and minimal detectable forces of 12 fN/sqrt(Hz). The cantilevers can be used in any conventional AFM setup using beam deflection. The low force noise and high force limit make encased cantilevers ideal for force spectroscopy applications.

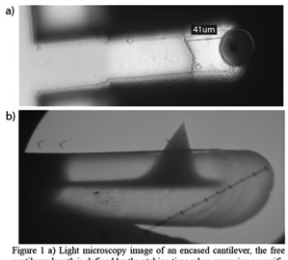

Figure 1 a) Light microscopy image of an encased cantilever, the free cantilever length is defined by the etching time when removing a sacrificial layer of Silicon Oxide. b) Transmission electron microscopy image showing the tip protruding from the encasement (Silicon Nitride).

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The Mechanism of the Formation of Nonadhesive Fibrinogen Matrices Examined by Atomic Force Microscopy

Ivan S. Yermolenko[1], Bryant Doss[2], Robert Ros[2], Tatiana P. Ugarova[1].
[1]Center for Metabolic and Vascular Biology, School of Life Sciences, Arizona State University, Tempe, AZ, USA, [2]Department of Physics, Arizona State University, Tempe, AZ, USA.

The plasma protein fibrinogen prevents adhesion of blood cells such as platelets and leukocytes to various surfaces, including fibrin gels. We have previously reported that the decrease in cell adhesion results from fibrinogen aggregation and the formation of an extensible multilayered matrix incapable of transducing strong mechanical forces through cellular integrins[1,2]. Here, using the various capabilities of Atomic Force Microscopy (AFM), we have examined the physical properties of matrices produced from human plasma fibrinogen (hFg) and two recombinant fibrinogens: recombinant normal fibrinogen (rFg) and fibrinogen with truncated αC domains (FgAα251). We have determined the thickness of matrices using AFM lithography, and also performed the high-resolution topographical visualization of single molecules within the matrices. With force spectroscopy, we have measured the extensibility, adhesion forces and the energy of AFM tip-fibrinogen matrix interactions. Our results indicate that hFg and rFg adsorbed at high concentrations form an extensible matrix containing 8-9 molecular layers. In contrast, FgAα251 forms only 2-3 molecular layers. These data indicate that the αC domains are critically involved in the formation of a fibrinogen multilayer. Furthermore, the inability of FgAα251 to form a thick multilayered matrix results in sustained cell adhesion, unlike hFg which completely prevents cell adhesion. To evaluate the contribution of the αC domains to the formation of multilayered matrices, we developed a model based on intermolecular domain interactions to simulate the adsorption process for hFg and FgAα251. These findings have implications for processes where deposition of fibrinogen occurs, such as thrombus formation and adsorption of fibrinogen on implanted biomaterials.

1. Podolnikova, N. P. *et al., Biochemistry,* **49,** 68-77 (2010).
2. Yermolenko, I. S. *et al., Langmuir,* **26,** 17269-17277 (2010).

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Total Internal Reflection Fluorescence Microscopy, Cell Adhesion, and Cell Traction Force Measurements on Soft Silicone Gels

Edgar Gutierrez, Alexander Groisman, Eugene Tkachenko.
University of California, San Diego, San Diego, CA, USA.

Substrate rigidity impacts cellular behaviors such as migration, gene expression, and cell fate. Total Internal Reflection Fluorescence (TIRF) microscopy enables selective visualization of the dynamics of substrate adhesions, vesicle trafficking, and biochemical signaling at the cell-substrate interface. Here we apply high-refractive-index silicone gels to perform TIRF microscopy on substrates with a wide range of physiological elastic moduli, measure traction forces exerted by cells on the substrate, and perform analysis of the cell spreading and the biochemical signaling that drives this process. We successfully implemented TIRF on mouse endothelial cells plated on ~30 micron thick layers of silicone gels with a refractive index of 1.49 and obtained high quality images. We also functionalized gel surfaces and covalently bound to them 40nm far-red fluorescent beads that were distributed nearly evenly with a high surface density. Measurements of displacements of the beads under cell traction forces made it possible to obtain high-resolution substrate deformation maps that were converted to traction force maps. We show mouse embryonic fibroblasts (MEFs) spreading over a significantly larger area on silicone gels of moderate physiological stiffness, 30 kPa, versus those grown on softer substrates of 0.5 and 3.5 kPa, in agreement with cells grown on polyacrylamide substrates of similar stiffness. We developed a novel microfluidic technique to measure the elastic moduli of thin gel layers and applied it to characterizing gels made using Sylgard 184 silicone with elastic moduli from 0.4 to 300 kPa. We prepared gel substrates with elastic moduli covering nearly the entire physiological range in different wells of a 6-well plate. Western-blot analysis of harvested cells revealed varying levels of phosphorylated Focal Adhesion Kinase (FAK) in MEFs grown on silicone gels with different elastic moduli.

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Natural Flexibility of Type I Collagen Molecules

Stephanie L. Holdener.
University of South Florida, Tampa, FL, USA.

Research concentrated around the extracellular matrix (ECM) and its tissue mechanics maintains prevalence in the biophysics area. A key player in the human connective tissue is type I collagen fibrils which make up a significant portion of the interstitial matrix, basement membranes, and the bone matrix. The formation of fibrils depends largely on individual collagen molecules and assumedly the amino acids that compose each helix in type I tropocollagen. Comprehensive examination of the flexibility within individual type I collagen molecules has not yet been completed. We have used atomic force microscopy to image and retrieve objective statistical data about deviations from the natural curvature in the molecule. This information will allow us to find the ranges in which the 'hinges' are occurring and determine if they are characteristic to the non-helical or helical domains. Additionally, we can utilize the information known about the sequence of type I collagen helices and the mechanical properties of the molecules to match the ranges to specific amino acids.

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The Effect of Tension on Closely Spaced Nicks in Naked DNA Molecules

Wei-Ju Chung, **Yujia Cui**, Ian C. Hsu.
National Tsing Hua University, Hsinchu, Taiwan.

Cells always protect their genomic integrity from environmental stresses and harmful chemicals produced during metabolism. Double-stranded breaks(DSB) in DNA caused by factors such as free radicals or ionizing radiation play an important role in triggering DNA repair pathways and apoptosis. These DSBs are often formed by closely spaced nicks. Although methods exist to quantify single nicks on plasmids, the detection of multiple nicks or clustered nicks on DNA molecules is not so straight forward. By stretching single DNA molecules in low ionic strength buffers, sections containing clustered nicks denature locally and induce DSB. The detection of closely spaced nicks on double-stranded (ds) DNA is done by applying tension on DNA molecules with dual-beam optical tweezers and notating the time at which the DNA breaks in relation to the starting time (sustaining time). The effects of nicks caused by the sample preparation process and oxidative agents in the buffer were investigated. DNA sustaining time under low force (4~6 pN) is around 50 minutes in low salt buffer. Higher forces (14~16 pN) cause the sustaining time to shift toward a shorter time frame (~30 minutes). The sustaining time of DNA molecules prepulled to a predetermined maximum force (14~16, 24~26, or 34~36 pN) also show shifts toward shorter time.

Emerging Single Molecule Techniques III

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Under-Filling Trapping Objectives Optimizes the use of Available Laser Power in Optical Tweezers

Mohammed Mahamdeh[1], Citlali Pérez Campos[2], Erik Schäffer[1].
[1]Biotechnology Center, TU Dresden, Dresden, Germany, [2]Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany.

For optical tweezers, especially when used in biological studies, optimizing the trapping efficiency reduces photo damage or enables the generation of larger trapping forces. One important, yet not-well understood, tuning parameter is how much the laser beam needs to be expanded before coupling it into the trapping objective. Here, we measured the trap stiffness for 0.5-2 μm-diameter microspheres for various beam expansions. We show that the highest overall trapping efficiency is achieved by slightly under-filling a high-numerical aperture objective when using microspheres with a diameter corresponding to about